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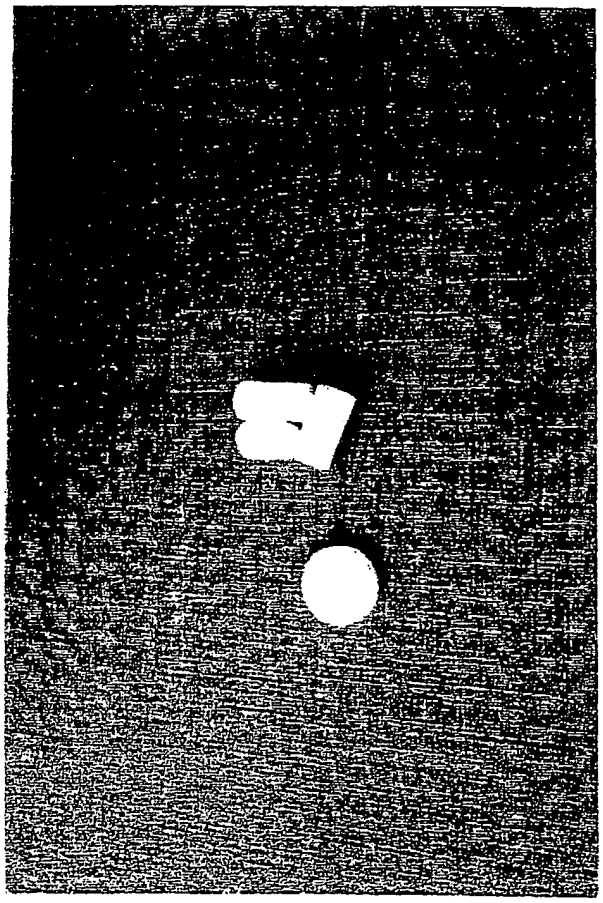
(54) Title: MINERAL-POLYMER HYBRID COMPOSITION



(57) Abstract: The present invention relates to self-setting compositions consisting in admixed liquid and solid components enable the formation of hardened bio-materials having a broad range of properties and performances. The present invention proposes a) a thermo-sensitive self-gelling liquid component, being water-based, comprising at least a polycationic and a phosphate source, wherein the liquid component is a thermo-gelling solution at a pH ranging from 6.5 to 7.4; b) a powder component consisting in at least two calcium phosphate sources. The preferred calcium phosphate source includes apatites, tricalcium phosphates, tetracalcium phosphates and dicalcium phosphates. Both solid and liquid components are admixed to form a flowable slurry that sets *in situ* into a hardened calcium phosphate based bio-material.

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MINERAL-POLYMER HYBRID COMPOSITION

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates generally to the preparation and use of an injectable self-setting mineral-polymer composition for repairing, replacing or therapeutically treating tissues and body parts. More particularly, the present invention includes the injectable self-setting mineral-polymer composition.

(b) Description of Prior Art

A large quantity of biomaterials has been introduced for hard-tissue repair and formation, including natural or synthetic materials, pure organic or inorganic materials, and organo-inorganic biohybrid or hybrid materials.

Conductive hard-tissue implants are passive biomaterials that provide a matrix to favor and support a new hard-tissue ingrowth and repair. They generally do not provide any osteogenesis property, in the meaning that such materials do not supply, by themselves, any osteogenesis or hard-tissue inductive factors, or any hard-tissue healing accelerators. Conductive structures have typically to favor the own ingrowth and reorganization of hard-tissues (Ex: osteoconductive materials).

The main constituent of hard-tissues is biological apatite that is commonly found in bone and teeth (65-98%). Calcium and phosphate ions are commonly contained in body fluids and mineral contents of hard tissues, including bones, dentine and dental enamel. They may also additionally contain other constituents such as carbonates, magnesium or sodium. Hydroxyapatite is generally recognized as being a calcium phosphate material with a crystal structure very close to biological apatite. Calcium phosphates, and some other ceramics, were found to be very useful biocompatible materials for hard-tissue repair. Today, a large family of ceramic biomaterials having different forms is available for repairing hard-tissues, and includes calcium phosphates, calcium carbonates, bioglasses and pure natural minerals.

difficult to shape, and consequently the interface between the bone tissue and ceramic implant is not perfectly continuous which may impair the osteoconduction efficiency. Calcium phosphate granules are currently produced with a wide size distribution, and available from 10 microns to 2.5 mm, but preferably used with a size between 90 and 400 microns. Granules can be injected, or at least administered through less invasive techniques, so as to fulfill the tissue defect. But granules have a mobility problem *in situ*, which limits their use and efficiency.

Ceramics such as calcium carbonates, coral or nacre are equally proposed under granular or block form, and present similar problems. Bioglasses are generally under granular or microspheric form (Bioglass®, USBiomaterials; Biogran®, Orthovita; Perioglass®).

Collagen, a component of soft- and hard-tissues, and Bone Demineralized Matrix (BDM) are the current organic materials for filling hard-tissue defects. Collagen was associated with mineral to form composite materials such as Collapat® or Collagraft® (NeuColl), Cerapatite-Collagen® (Ceraver-Osteal), Ossatite® composite (MCP). Polymeric materials such as polylactic acid, polyglycolic acid, polylactic-co-glycolic acid microspheres, etc were also proposed for bone defect filling and repair, but are less current than calcium phosphate granular materials. One new development is Immix® (Osteobiologics) bone-grafting material based on PLA/GA.

Injectable Bone Substitutes

Inorganic or organo-inorganic bone cements and/or remineralizing systems form another family of promising injectable self-setting or self-hardening osteoarticular materials. Self-setting cements were typically composed of a solid mineral component mixed with a liquid component. Solid mineral components generally contain calcium phosphates, such as monocalcium phosphates [$Ca(H_2PO_4)_2.H_20$], dicalcium phosphates [$CaHPO_4$, $CaHPO_4.2H_2O$], tricalcium phosphates [α-$Ca_3(PO_4)_2$, β-$Ca_3(PO_4)_2$] and tetracalcium phosphates [$Ca_4(PO_4)O$], with or without other calcium sources and/or phosphate sources, calcium carbonates and/or organic or inorganic additives.

Calcium phosphate remineralizing and cement systems differ by the liquid to solid ratio. Cements are produced from calcium phosphate

dicalcium phosphate that does not self-harden, and has insufficient remineralizing capacity.

Brown and Chow (US Patent Nos. 4,612,053; 4,518,430; and Re33,221) proposed a self-setting composition based upon an aqueous mixture of tetracalcium phosphate (TTCP) with at least another calcium phosphate component in excess, selected from dicalcium phosphate or brushite, tricalcium phosphates and modified tricalcium phosphates, octacalcium phosphate [$Ca_8H_2(PO_4)_6.5H_20$] which was able to self-harden into a cement at an ambient temperature. Additional calcium or phosphate sources consisted mainly in $CaCl_2$, $Ca(C_2H_3O_2)$, NaH_2PO_4 and $NH_4H_2PO_4$. The slurry containing calcium phosphates in excess had a pH in the vicinity of 7.4. This cement paste was proposed first for dental restorative applications although many orthopedic indications were proposed. Later, Chow and Takagi (US Patent No. 5,545,254) showed that the preparation of TTCP free of surface calcium oxide or hydroxyapatite greatly improved the quality of such bone cements for dental and orthopedic applications. Remineralization and cement compositions were biocompatible precursors of hydroxyapatite, having two properties: a) they were self-hardening and form materials with sufficient strengths for medical and dental applications; b) they were resorbed *in situ* and progressively replaced by new hard-tissues.

Liu and Chung (US Patent No. 5,149,368) proposed other TTCP-based cement formulations where TTCP was admixed with water and acidic citrate to form a paste having a pH greater than 5. The weight ratio of powder to liquid was between 2:1 and 15:1. Constantz et al. (US Patent No. 5,053,212) developed a composition precursor of hydroxyapatite by admixing a calcium source with an acidic phosphate source. In the preferred embodiment, TTCP was mixed with calcium oxides, calcium carbonates (typically $CaCO_3$), monocalcium phosphate monohydrate (MCPM) and/or orthophosphoric acid. Calcium to phosphate ration was about 1.25-2.0 to 1.0. Later, another bone cement was described where a dry component was admixed with a compatible lubricant and an anti-microbial agent (US Patent No. 5,968,253). Its dry component was made of reactive α-tricalcium phosphate (60-95% dry wt.), monocalcium phosphate monohydrate (1-20% dry wt.) and calcium

was between 0.2 and 0.5. This cement gave a crystalline hydroxyapatite biomaterial with a compressive strength about 15 to 25 MPa.

A calcium orthophosphate composition that hardens in 100% humidity environments into a calcium phosphate cement was composed of a mixture of three to four calcium sources with water. The composition had a pH ranging between 6.5 and 8.0. Calcium sources were selected preferably among monocalcium phosphate monohydrate (MCPM), dicalcium phosphate or brushite, tricalcium phosphates, and modified tricalcium phosphates, octacalcium phosphate, apatites, and other calcium compounds such as $Ca_{8.5}Na_{1.5}(PO_4)_{4.5}(CO_3)_{2.5}$, $Ca_9(PO_4)_{4.5}(CO_3)_{1.5}$, $Ca_4Mg_5(PO_4)_6$, $CaZn_2(PO_4)_2$, $CaKPO_4$, $CaNaPO_4$, $Ca_{10}Na(PO_4)_7$, Ca_2PO_4Cl, CaO, $Ca(OH)_2$, $CaMgO_2$ and $Ca_{10}(PO_4)_6Cl_2$.

Basic calcium phosphate cements self-setting in hydroxyapatite were developed by Chow and Takagi (US Patent Nos. 5,525,148 and 5,954,867). Liquid components contained liquid phosphate component having a pH above 12.5 (phosphate > 0.2 mol/l). Solid calcium phosphate component had a Calcium to Phosphate between 3.0 and 5.0, included various calcium phosphates, except TTCP, and a calcium source. Proposed calcium phosphates were dicalcium phosphates, tricalcium phosphates, octacalcium phosphate and/or amorphous calcium phosphate. Additional sources of calcium were selected typically among calcium carbonates, calcium oxides, and calcium hydroxides. Additional minerals were also added in minor concentrations. The pH of the composition was potentially adjusted above 12.5 by adding sodium hydroxide.

Commercial developments in calcium phosphate bone cements are given by SRS® (Norian), BoneSource® (Stryker/Howmedica), alpha-BSM® (ETEX Corp.), all three giving carbonated apatite *in situ*, and Cementek® (Teknimed SA).

Most common calcium phosphates in self-setting cements were selected from monocalcium phosphate monohydrate [$Ca(H_2PO_4)2.H_20$], dicalcium phosphate (DCP) or brushite [$CaHPO_4$, $CaHPO_4.2H_2O$], tricalcium phosphate (TCP) [α-$Ca_3(PO_4)_2$, β-$Ca_3(PO_4)_2$], tetracalcium phosphate (TTCP)[$Ca_4(PO_4)O$], amorphous calcium phosphate

Incorporation of polymer in cement composition was proposed to give some specific properties: a) to improve the handling properties and wettability of the cement; b) to avoid the cement composition to disintegrate in aqueous media such as the physiological fluids, and allow to pre-shape the composition; as a consequence, this reduced the need for removal of body fluid, hemostasis, or the like.

Polyacid or polyol polymers, polysaccharides and polypetidics were preferentially chosen for incorporation in calcium phosphate cement compositions. Polycarboxylics (polycarboxylic acid), poly(ethylene glycol), poly(propylene glycol), methyl cellulose, poly(vinyl alcohol), carboxymethyl cellulose, hydroxypropyl methylcellulose, and the like, were proposed as polymeric components. Collagen was optionally introduced in a cement composition by Constantz et al. (US Patent No. 5,053,212). Chitin, chitosan, starch, gum, pectic acid, alginic acid, hyaluronic acid, chondroitin sulfuric acid, dextran sulfuric acid and their salts were reported as potent polysaccharide ingredient (US Patent Nos. 5,152,836; and 5,980,625; and European patent application publication No. EP-899,247 A1).

Chitosan was admixed in many liquid components of calcium phosphate cement compositions. Chitosan in citric, malic, or phosphoric acid aqueous medium was the liquid component of a self-setting TCP or TCP/TTCP cement (US Patent Nos. 5,281,404 and 5,180,426). Chitosan in bone cements or substitutes was also studied in the scientific literature, as reported by Leroux et al. (*Bone*, Vol. **25**, No 2, supplement, 1999:31S-34S), Hidaka et al. (*J. Biomed. Mat. Res.*, **46**:418-423, 1999), Ito (*Biomaterials*, **12**:41-45, 1991). It has also been reported the use of chitosan in calcium phosphate compositions. Typically, chitosan 0.05% wt. in an acidic aqueous medium (acid 25-55% wt.) was used as lubricant for a solid component consisting in a mixture of TCP and TTCP. Chitosan was chosen to prevent the powder dispersion and cement disintegration.

Osteoconduction and osteogenic performances of chitosan based materials were reviewed, and applied to biomaterials development. Chitosan with immobilized polysaccharides such as heparin, heparan sulfate, chondroitin sulfate and dextran sulfate was reported for stimulating hard-tissue regeneration by Hansson et al. (International Patent Application publication WO96/02259). Osteoinductive compositions were

phosphates and monocalcium phosphates,

wherein when said components of step a) and b) are intimately and uniformly mixed together, said components of step a) and b) form an injectable thermo-setting slurry, said slurry when heated turns into a solid material.

The cationic polymer may be a polysaccharide, a polypeptide or a synthetic polymer. The cationic polymer may have a concentration in said liquid component between 0.1 and 5.0% wt. The cationic polymer is preferably chitosan or collagen, or a mixture of chitosan and collagen. The cationic polymer can also be a partially-deacetylated chitin or chitosan with a degree of deacetylation between 30 and 99%. The cationic polymer can further be a polylysine.

The monophosphate salt may have a basic character.

The liquid component can comprise a first phosphate source selected from the group consisting of $Na_2PO_4C_3H_5(OH)_2$, $Fe_2PO_4C_3H_5(OH)_2$, $K_2PO_4C_3H_5(OH)_2$, $MgPO_4C_3H_5(OH)_2$, $MnPO_4C_3H_5(OH)_2$, $Ca_2PO_4C_3H_5(OH)_2$, $Na_2PO_7C_3H_7$, $Na_2PO_7C_4H_7$, $K_2PO_7C_4H_7$, $NaPO_7C_4H_8$, $K_2PO_7C_4H_8$, $Na_2PO_8C_5H_9$, $K_2PO_8C_5H_9$, $NaPO_8C_5H_{10}$, $KPO_8C_5H_{10}$, $Na_2PO_9C_6H_{11}$, $NaPO_9C_6H_{12}$, $K_2PO_9C_6H_{11}$, $KPO_9C_6H_{12}$, $Na_2PO_8C_6H_{13}$, $K_2PO_8C_6H_{13}$, $NaPO_8C_6H_{14}$, $KPO_8C_6H_{14}$, $Na_2PO_9C_6H_{12}$, $K_2PO_9C_6H_{12}$, $NaPO_9C_6H_{13}$, $KPO_9C_6H_{13}$, $Na_2PO_8C_{10}H_{11}$, $K_2PO_8C_{10}H_{11}$, $NaPO_8C_{10}H_{12}$, $KPO_8C_{10}H_{12}$ and the like, or a derivative thereof.

The monophosphate salt is a sodium, magnesium, potassium, ferric and/or calcium alpha- or beta-glycerophosphate salt, or a mixture thereof.

The monophosphate salt may be glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate or fructose-6-phosphate salt, or a mixture thereof.

The liquid component preferably has a pH between 6.8 and 7.2 and a viscosity superior to 200 mPa.s.

The liquid component can further comprise at least one other water-soluble polymer selected from the group consisting of polypeptides, cellulosics and synthetic polymers, including methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl propylcellulose,

$Ca_9(PO_4)_{4.5}(CO_3)_{1.5}$, and the like.

The powder component preferably comprises a strontium salt including strontium carbonate or at least one calcium phosphate selected from the group consisting of fluoride, strontium, carbonate, magnesium, zinc, and barium containing calcium phosphates.

The powder component can comprise at least one inorganic salt including sodium phosphates and disodium glycerophosphate, or the like, or alternatively, it can comprise at least one organic salt including oxalate, citrate, malate, gluconate, lactate, lactobionate, or the like.

The powder component can comprise at least one organic salt including oxalic, citric, malic, gluconic, lactic, lactobionic acids, or the like.

The powder component is preferably a powder having a size ranging from 0.1 to 100 micrometers.

Preferably, the composition further comprises a bioactive ingredient such as a drug, a protein, a peptide, a synthetic molecule or an inorganic molecule, or it can comprise at least one osteoinductive agent selected from the group consisting of hormones, bone proteins and mixtures of osteoinductive proteins, demineralized bone matrix (DBM) or powder (DBP), bone morphogenic proteins (BMP), sialoproteins, osteonectin, osteopontin, osteocalcin, calcitonin. Preferably, the composition further comprises at least one growth factor selected from the group consisting of IGF, EGF, a-FGF, b-FGF, PDGF-A, PDGF-B and TGF-beta.

The composition can also further comprise an antiresorptive, antibiotic, antiviral, antitumor, or an immunosupressive agent.

In accordance with the present invention, there is also provided the use of the composition as defined above for injection into a defect, cavity or interface of a body's tissue, said composition setting *in situ* into a hardened filling material, or for the manufacture of a medicament for injection. The composition may be injected into a defect, cavity or interface of a cancellous, cortical or corticocancellous bone. The composition may also be injected into the metaphysis or diaphysis of a bone or into a fractured bone, between the bone fragments of fractured bone. The composition once injected sets *in situ* into a filling hardened material.

injectable thermo-setting slurry, said slurry once heated setting into a solid material.

Preferably, the inorganic salt is selected from carbonate, phosphate, strontium, fluoride salts, and the like, and the organic salt is preferably selected from citrate, malate, lactate, gluconate salts, and the like.

The organic acid can be selected from citric acid, malic acid, lactic acid, gluconic acid, and the like, and the organic compound can be selected from the group consisting of biological fluids and components, water-soluble or miscible organic polyols, drugs, amino-acids, proteins, and the like.

The composition can also further comprise a water-soluble or miscible organic polyol, including sugar-polyol, saccharide-polyol and glycol, selected from the group consisting of glycerol, mannitol, sorbitol, ethylene glycol oligomers, propylene glycol oligomers, saccharose, fructose, glucose, maltose, and the like.

The composition can still comprise glucosamine and/or histidine.

The composition can further comprise a strontium containing compound, a carbonate containing compound or a fluoride containing compound.

Also in accordance with the present invention, there is provided a method of preparation of an injectable self-setting composition, said method comprising the step of admixing a water-based liquid component comprising at least one cationic polymer and one mono-phosphate salt with a powder component comprising at least two calcium phosphate sources selected from apatites and apatitic calcium phosphates, octacalcium phosphates, amorphous calcium phosphates, tetracalcium phosphates, tricalcium phosphates, dicalcium phosphates and monocalcium phosphates, wherein said liquid component comprising at least one cationic polymer and one mono-phosphate salt; said liquid component having a pH ranging from 6.5 to 7.4, said liquid component having an endothermally gelling character and being free of insoluble particles, said admixing thus forming an injectable thermo-setting slurry, said slurry when heated turns into a solid material.

In one embodiment of the invention, there is provided a method

In accordance with the present invention, the composition comprises a liquid component and a solid component, such components being intimately mixed together; said liquid component being endothermally sensitive as previously defined.

The term "mineral-polymer hybrid" refers herein to a biphasic system where a mineral component is associated to a polymer component, whatever said mineral and polymer components are liquid or solid.

The term "liquid component (or phase)" refers herein to the component that is a water-based solution, and particularly a water-based polymeric solution.

The term "powder component (or phase)" refers herein to the component that is a solid material, said solid materials being preferably a powder, particulate or granular material. Also used for solid component is "mineral component or phase".

The term "dry ingredient" refers to a dry solid material that enters in the preparation of the solid component and mineral-polymer hybrid composition. In most cases, it is a mixture of solid particulates made of minerals or organics.

"Apatitic" refers herein to a compound that has mainly an apatite-related crystallographic phase.

"Bioactive agent" refers herein to a substance that presents an established biological activity of interest for the use of the hybrid composition. "Non-bioactive agent" corresponds to a substance used without any consideration for a possible biological activity of the hybrid composition.

"Self-setting" refers herein to a reaction that occurs in the hybrid composition between the components of the liquid and solid components. It is basically a dissolution and reprecipitation of the minerals of the solid component in the liquid component. It results in macroscopic and characteristics changes of the hybrid composition.

"Self-hardening" refers herein to the formation of a continuous solid material or network within the hybrid composition. This material or network is built from minerals, but may incorporate organic (mineral-polymer). Self-hardening excludes self-gelling. Self-hardened materials are not highly hydrated, and do not correspond to gels.

In one embodiment, a self-setting mineral-polymer composition comprises a thermo-gelling liquid component and a mineral powder component that gives a self-setting (self-hardening) composition at the body temperature. Various composition of the present invention can be self-hardening to various levels, which give potent high strengths, mainly ceramic-composed, solid bio-materials with a plain or porous structure.

The composition of the present invention is preferably used with hard-tissues of the body, typically bone, dentine and enamel.

Preparation of the liquid component

In the present invention, the liquid component is an endothermally sensitive solution, and consists in a polymeric aqueous solution. In a preferred embodiment, the liquid component comprises water, and an acid-soluble organic and/or inorganic acid, at least one acid-soluble cationic polymer, and at least one water-soluble phosphate source. In other embodiments, the composition comprises water, and an acid-soluble organic and/or inorganic acid, at least one acid-soluble cationic polymer, at least one of a water-soluble phosphate, and one of water-soluble sulfonate and carboxylate salt. The acid-soluble cationic polymer is defined as being a hydrophilic cationic polymer that is soluble in an acidic aqueous medium with a pH inferior to 6.5.

The liquid component is characterized by its endothermal sensitivity which means generally that it presents a sol-gel transition temperature (SGTT), a liquid state (sol state) at a temperature lower than the SGTT, and a gel state comprising a gel which is substantially water-insoluble at a temperature higher than the SGTT.

In the liquid component, the acid-soluble polymer is dissolved by using organic and/or inorganic acids, including malic acid, propionic acid, phosphoric acid, glycerophosphoric acid, orthophosphoric acid, lactic acid, hydrochloric acid, ascorbic acid, formic acid, acetic acid, and the like. The polymer is dissolved in an acidic aqueous medium having a pH ranging between 1.0 and 5.0, preferentially between 1.0 and 4.0. The acid-soluble cationic polymer is a hydrophilic polysaccharide, including partially deacetylated chitins and chitosans, and an amino-substituted polysaccharide having the desired properties. It can also be an amino-substituted dextran. The acid-soluble cationic polymer can also be a

thereof or a mixture thereof. Ideally, the phosphate source is alpha- or beta-glycerophosphate (glycerol-2-phosphate, glycerol-2-phosphate), glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate or fructose-6-phosphate disodium or dipotassium, magnesium, or a mixture thereof.

The liquid component may optionally comprise at least one sulfonate source, in a proportion of 0.1 to 10% w/v, selected among N-[carbamoylmethyl]-2-aminoethane sulfonate (ACES), N,N-bis[2-hydroxyethyl]-2-aminoethane sulfonate (BES), 3-[N,N-bis(2-hydroxyethyl)amino]-2-hydroxy propanesulfonate (DIPSO), N-[2-hydroxyethyl]piperazine-N'-3-propane-sulfonate (EPPS), N-[2-hydroxyethyl]piperazine-N'-3-propanesulfonate (HEPES), 2-[N-morpholino]ethanesulfonate (MES), 4-[N-morpholino]butane-sulfonate (MOBS), N-tris[hydroxymethyl]methyl-2-minoethanesulfonate (TES), and the like, or a mixture thereof.

The liquid component may optionally comprise other molecules such as water-soluble molecules having one acid and at least two amino groups, or more amino groups than acid groups, or at least one amino groups and multiple alcohol groups; said molecules having a moderate basic character and a pKa between 6.0 and 7.4. This molecule is generally selected among amino-acid residues or sequences, including histidine (HIS) or lysine (LYS) residues or sequences, and/or among a group comprising bis[2-hydroxyethyl] iminotris[hydroxymethyl] methane (BIS-TRIS), Tris[hydroxy-methyl] amino-methane (TRIZMA), and the like, or a mixture thereof.

All proposed liquid components have a pH ranging between 6.5 and 7.4, an intrinsic viscosity ranging between 5 and 100,000 mPa.s at 21°C. All liquid components, being endothermally sensitive, have a sol-gel transition temperature, and form homogeneous solid aqueous gels at a temperature, between 15°C and 60°C, preferably between 25°C and 45°C, and more preferably at 35-40°C.

Other organic compounds being non-bioactive may be admixed to the liquid component so as to provide specific chemical or physical properties. Representative compounds include sugar-polyols such as glycerol, mannitol or sorbitol, and saccharide-polyols such as fructose,

phosphates with Ca/P molar ratio in the range 1.66 to 1.5 or less. Compounds including interlayers of octacalcium phosphate are included.

The calcium phosphates can be selected in a group comprising $Ca(H_2PO_4)_2.H_2O$, $CaHPO_4.2H_2O$, $CaHPO_4$, $CaZn_3(PO_4)_2$, $CaZnPO_4$, $CaNaPO_4$, Ca_2PO_4Cl, alpha-$Ca_3(PO_4)_2$, beta-$Ca_3(PO_4)_2$, $Ca_3(PO_4)_2.H_2O$, $Ca_4(PO_4)_2O$, $Ca_8H_2(PO_4)_6.5H_20$, $Ca_9(HPO_4)(PO_4)_5OH$, $Ca_{10}(PO_4)_6(OH)_{2-2x}O_x$, $Ca_{10}(PO_4)_6(OH)_2$, and derivatives thereof. Calcium phosphate sources may comprise natural mineral components including hard-tissue, enamel or dental apatite, coral or nacre. Calcium phosphate sources may also be selected among apatitic and nonapatitic calcium phosphates containing fluoride, strontium or carbonate (calcium fluoride phosphates, calcium strontium phosphates, carbonated calcium phosphates, fluorinated and carbonated calcium phosphates, fluorinated calcium strontium phosphates, fluorinated and carbonated calcium strontium phosphates, and the like).

The solid component may also comprise a phosphate source such as a sodium phosphate compound.

The solid component may also comprise a calcium source selected in a group comprising CaO, $Ca(OH)_2$, $CaCO_3$, $CaCl_2$, $CaMgO_2$, CaF_2, $CaPO_4C_3H_5(OH)_2$, $Ca(H_2PO_4)_2.H_2O$, $CaHPO_4.2H_2O$, $CaHPO_4$, alpha-$Ca_3(PO_4)_2$, beta-$Ca_3(PO_4)_2$, $Ca_3(PO_4)_2.H_2O$, $Ca_4(PO_4)_2O$, $Ca_8H_2(PO_4)_6.5H_20$, $Ca_9(HPO_4)(PO_4)_5OH$, $Ca_4Mg_5(PO_4)_6CaO$, $Ca_{10}(PO_4)_6Cl_2$, $Ca_{10}(PO_4)_6(OH)_{2-2x}O_x$, $Ca_{10}(PO_4)_6(OH)_2$, and derivatives thereof.

The solid component may comprise other mineral ingredients such as a carbonate, strontium, fluoride, magnesium, zinc or barium source, or other minerals. Carbonates may be typically selected among Na_2CO_3, $CaCO_3$, K_2CO_3, $MgCO_3$, $ZnCO_3$, $Ca_9K(PO_4)_5(CO_3)_2$, $Ca_{8.5}Na_{1.5}(PO_4)_{4.5}(CO_3)_{2.5}$, $Ca_9(PO_4)_{4.5}(CO_3)_{1.5}$, and the like. The fluoride source may be selected among NaF, Na_2Si_6F, KF, KSi_6F, CaF_2, MgF_2, ZnF_2, and the like. Strontium compounds may be strontium salts (strontium chloride, oxides and the like), strontium phosphate salts. Other ingredient may be oxides and/or hydroxides such as MgO, $Mg(OH)_2$, ZnO, and the like.

eliminated from this liquid. Identically, the dry mixture is preferentially stored under strict anhydrous conditions so as to avoid any contamination or cross-reaction with water. Solid additives, being organic or inorganic, may be admixed with the dry ingredients at the dry mixing step. Incorporation of bioactive agents in the solid component may be performed during the dry mixing, or later, during a second mixing step.

Preparation of self-setting hybrid compositions/bio-materials

In the present invention, self-setting compositions are prepared by intimately mixing the liquid component and the powder component. Mixing may be performed manually by kneading, or physico-mechanically by using homogenizers, mixers or mills. There is no special preference for the mixing instruments, but the composition must be as uniform and homogeneous as possible. A specially designed instrument may be used as well as to mix and deliver the composition before use.

The liquid component is one selected among those previously described. One preferred basic liquid components comprise water, acid, chitosan and a first source of phosphate, glycerophosphate. Acid is generally selected among hydrochloric acid, glycerophosphoric acid, phosphoric acid, citric acid, acetic acid, lactic acid, and the like. The starting acidic aqueous medium is generally a 0.05 to 1N acid/water solution, and preferably a 0.05 to 0.5N solution. Chitosan is generally selected among partially N-deacetylated poly(glucosamine) having a deacetylation degree between 60 and 100%, preferably between 30 and 99% and more preferentially between 84 and 98%. It is present in the liquid component at a concentration ranging from 0.1% to 10% w/v, and more preferably between 0.1% and 2.0% w/v. The source of phosphate is generally an organic monophosphate dibasic salt, such as glycerol-2-phosphate and/or glycerol-3-phosphate sodium or magnesium salts, at a concentration between 0.1% and 20% w/v, and ideally between 1.0% and 10% w/v. The pH of the liquid component varies between 6.5 and 7.4, and preferably between 6.8 and 7.2. The viscosity of the liquid component is ranging between 5 mPa.s to 100,000 mPa.s, and preferably between 10 mPa.s and 1,000 mPa.s. As previously described, additional reagents may be an organic monosulfonate salt and/or a second hydrophilic polymer, and/or an organic molecules, and/or a bioactive agent. The liquid

component also comprises an organic compound, being not an acid or a salt, such as an amino-acid, a polyol, a sugar, and the like.

In one embodiment, the solid component comprises a dry mixture of alpha-tricalcium phosphate and calcium deficient apatite. In another embodiment, the solid component comprises a dry mixture of alpha-tricalcium phosphate and calcium deficient apatite with at least one of an organic acid, an organic salt, an organic (nonacid, nonsalt) compound and a noncalcium phosphate salt.

The mineral ingredients of the solid component are dry-mixed as previously described so as to obtain a homogeneous dry mixture. This dry mixture can be performed in several distinct sequences. Preferentially, the dry minerals or ingredients were mixed-milled in a ball mill so as to obtain a mineral powder mixture homogeneous and of the adequate size. This mixing/milling may be performed in acetone or hexane solvent to avoid any moisture. An appropriate size of dry ingredient ranges between 1 and 250 µm, generally between 1 and 50 µm, and preferably between 1 and 20 µm.

The calcium to phosphate ratio of the solid component generally varies between 1.0 and 4.0, and typically between 1.0 and 2.0, and preferably between 1.2 and 1.8.

The mixing of liquid (L) and solid (S) components is performed at a liquid/solid weight ratio between 0.05 and 1.50 (mL/g), and preferably between 0.2 and 1.0 (mL/g).

The liquid component is endothermally sensitive, but the resulting paste is self-setting with time, thus self-hardening at 37°C and 100% humidity into a solid bio-materials that looks like a ceramic or cement-like materials. The cement-like materials have higher compression strengths: after two days ageing in a water solution, the wet ultimate compression strength reaches typically 5-10 MPa and up. Resulting mineral composition includes apatite. The material is resorbable *in situ* over a period of 18 months.

Bioactive ingredients

Any bioactive ingredients may be incorporated and released from self-setting compositions and bio-materials. The incorporation can be assessed out via the liquid or solid component. Bioactive agents include drugs, therapeutic agents, osteogenic agents and anti-resorptive agents.

More generally, the compositions may be useful for all repair, regeneration, filling, replacement procedures associated to hard-tissues as well as for delivering drugs or bioactive agents to hard-tissues.

The composition can be injected to the filling and repair of internal bone cavity, of local treatment of osteoporotic bones, and other demineralized bones and demineralization disorders; of bone defects or cavities, for example in the case of periodontal defects with bone loss, augmentation of the alveolar ridge or surgically-performed hard-tissue defects following resection of diseased hard-tissue parts; of bone fractures for repairing fractures, fixing bone fragments, delivering agents that accelerate the sequence of fracture healing.

Self-setting mineral-polymer compositions are to be used in orthopaedic, cranio-maxillofacial or dental surgery.

The present invention will be more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I
Preparation of Liquid Phases

The liquid phase of bone composition is an endothermally self-forming aqueous solution made of one hydrophilic biopolymer and at least one water-soluble phosphate source.

A representative liquid phase is a chitosan/glycero-phosphate [chitosan-GP] aqueous solution. An acidic chitosan aqueous solution (2.0% w/v) was made with a chitosan previously deacetylated at 83-97%, filtered and dialyzed, and was prepared from a 0.097M (0.10M) HCl solution. A chitosan/glycerophosphate aqueous solution was prepared from the 2.0% (w/v) chitosan in HCl aqueous solution and a 8.4% (w/v) disodium glycerophosphate in distilled water solution. Final concentrations (w/v) in the self-gelling chitosan/glycerophosphate systems was approximately 1.6-2.0% (chitosan) and 6.75-8.2% (glycerol-phosphate).

Glycerophosphate salts act herein as buffering/thermo-gelling agents for the chitosan solution. Other buffering/ thermogelling phosphate sources may be used, typically organic monobasic phosphate salts, such as glucose-phosphate or fructose-phosphate salts. Other buffering agents

found to affect the gelling or precipitating temperature of the polymer, thus leading to a precipitation of the chitosan/ GP/polymer(2) system.

Table 2

Liquid phase compositions having an admixed second water-soluble polymer

Polymer (2)	Polymer content (w/v)	Chitosan content (w/v)	GP content (w/v)	Remarks
Hydroxyethyl Cellulose	1.0%	2.0%	8.0%	Form gels.
Hydroxypropyl Methyl Cellulose	0.55%	1.0%	4.5%	Form gels.
Polyethylene glycol	1.0%	2.0%	8.0%	Form gels.
Methyl Cellulose	1.0-2.0%	1.0-2.0%	4.0-6.0%	Form gels. Precipitation may occur with higher GP contents.
Pluronic®	1.0%	2.0%	2.0%	Form gels. Precipitation may occur with higher GP contents.
Collagen (type I)	1.0%	1.0%	8.0%	Form gels.

All presented concentrations (%, mol/l...) are final.

All polymers (2) were dissolved in a prepared chitosan-GP solution, except for collagen, and more generally polyamines, that are dissolved in combination with the chitosan.

b) **Addition of water-soluble ingredients of interest**

Some organic molecules that are soluble or miscible with water may be added to the chitosan-based liquid phase to give modified or improved physico-chemical characteristics, mechanical or handling performances, or biological properties. This includes polyols, sugars, amino-acids, polysaccharides, and other biochemicals.

Glycoaminoglycans may be added to the chitosan-GP solution to a certain extent. It must be taken care of not inducing precipitation of chitosan. Heparin (see Table 4) was used as the GAGs to be added. Chitosan solutions were 4.0% w/v chitosan (deacetylation 95%) in 0.19M HCl. GP solutions were 54,6 % w/v in water. Heparin in water solutions was at 1 mg/mL (A), 0.1 mg/mL (B), 10 µg/mL (C) and 1 µg/mL (D).

Table 4

Liquid phase compositions having added water-soluble non-polymeric ingredient (Heparin)

#	Composition	pH	Remarks
1.	500 µL chitosan + 150 µL GP 250µL water + 100 µL Heparin (B)	7.04	Gels.
2.	500 µL chitosan + 250µL water 150 µL GP + 100 µL Heparin (C)	7.01	Gels.
3.	500 µL chitosan + 250µL water 150 µL GP + 100 µL Heparin (B)	6.87	Gels.
4.	500 µL chitosan + 250µL water 150 µL GP + 100 µL Heparin (A)	6.97	Reduced precipitation. Gels.

c) Addition of a Second water-soluble Phosphate source to the Liquid Phase

An acidic chitosan aqueous solution (2.0-4.0 w/v) was made with a chitosan deacetylated at 83-85%, filtered and dialyzed, and was prepared from a 0.1M HCl solution (see Table 5).

phosphate solution (1) (80:20 to 50:50, vol) showed no signs of precipitation, and gelled within 30 minutes at 37°C.

Table 6

Composition of liquid phases supplemented with a second source of water-soluble phosphate

Composition	pH	Chitosan:GP (% w/v)	[Phosphate] mol/l	Gelling time (initial)	Precipitation
10 ml chitosan-GP	7.0	4.0:8.2	0	30 minutes	No
9 ml chitosan-GP + 1 ml phosphate (3)	6.7	3.6:7.4	0.05		More turbid
8 ml chitosan-GP + 2 ml phosphate (3)	6.7	3.2:6.6	0.1		Highly turbid; Gels heterogeneously

A concentrated phosphate solution (2) was prepared from 283.92 g/l of Na_2HPO_4 (0.2 mol/l disodium hydrogen phosphate) and 239.96 g/l of NaH_2PO_4 (0.2 mol/l sodium dihydrogen phosphate) and had a pH of 7.4 at 37°C. This phosphate solution was used with dilutions at 1:1, 1:10, 1:100 and 1:1000. Equal volumes (50:50) of the diluted to concentrated phosphate solution (2) and chitosan-GP solution were mixed homogeneously. The pH of the resulting solutions was measured, and the solutions disposed at 37°C for gelation, all signs of precipitation being noted. All chitosan-GP/phosphate (2) gelled with various rates at 37°C.

A more concentrated phosphate solution (3) was prepared: 0.5 mol/l NaH_2PO_4 (600 g/l) and 0.5 mol/l Na_2HPO_4 (709.8 g/l). Volumes of the concentrated phosphate solution (3) was added to chitosan-GP solutions, and mixed homogeneously. The pH of the resulting solutions was measured, and the solutions disposed at 37°C for gelation, all signs of precipitation being noted. Chitosan-GP is fully compatible with 5 mM PBS solution at pH 7.2-7.4. Compatibility depends upon the phosphate content (Tables 2-4): the addition of highly concentrated phosphate solutions

e) Typical preparation of sterile Liquid Phase

Sterile Liquid phases

Sterilization of liquid phase can be performed during the preparation and processing of the chitosan-GP solutions. The chitosan-GP systems can not be sterilized by energizing methods, due to unexpected and undesirable thermal gelling. Chitosan solutions (no GP) and GP solutions (no chitosan) are to be sterilized separately. GP aqueous solutions have no viscosity and are sterilized by filtration in all cases, without any noticeable adverse effects. Chitosan materials (solid) or chitosan solutions (acidic aqueous medium) must be sterilized while avoiding the occurrence of degradative effects on both chitosan polymer and chitosan-GP systems. Table 8 illustrates the effects of sterilizing on Chitosan-GP systems (no additive).

Table 8

Effects Of Sterilizing On Chitosan-Gp Systems (No Additive)

Chitosan sterilization	Effects on chitosan biopolymer	Effect of thermogelling Chitosan-GP systems.
Autoclaving of chitosan solutions in acidic media.	Controlled modification;	Gels (slightly decreased gelling rate).
Autoclaving of chitosan suspension in water.	Controlled modification;	Modify gel properties.
Irradiation of chitosan materials (4°C).	Controlled modification;	Gels (slightly decreased gelling rate).
Irradiation of chitosan materials (20°C).	Stronger modification;	Gels (affect the gelling rate).

EXAMPLE II
Cement-like compositions and bio-materials

Compositions for cement-like bio-materials were prepared from liquid and solid (mineral) phases, liquid phases being prepared typically as described in Examples 1 and being thermally sensitive. The solid phase is a powder phase, generally containing minerals such as calcium

Table 9

Composition of liquid and solid phases for TCP/MCP/CC based cement-like bio-materials

Mineral Phase	Ca/P	Liquid Phase	L/S ml/g	Observations
βTCP/MCPM	1.45	Water	0.6	Set.: 3h; hard.: 4 (24h)
		Gelling CGP (1% C)	0.6	Set.: 3h; hard.: 4 (24h)
βTCP/MCPM	1.45	NaPO4 0.01M	0.45	Set.: 1 h; hard.: 5 (20h)
		Gelling CGP (1% C), NaP 0.01M	0.45	Set.: 1 h; hard.: 4 (20h)
βTCP/MCPM/ HAP	1.59	Gelling CGP (1% C)	1	Set.: 4 h; hard.: 2 (24h)
		Gelling CGP (1% C), NaP 0.01M	1	Set.: 2 h; hard.: 3 (24h)
βTCP/MCPM/ $CaCO_3$	1.59	Gelling CGP (1% C)	0.40	Set.: 1 h; hard.: 5 (24h)
		Gelling CGP (1% C), NaP 0.01M	0.40	Set.: 1 h; hard.: 5 (24h)
βTCP/MCPM	1.33	Water	0.3	Set.: 30 s; hard.: 5 (2min)
		Gelling CGP (1% C)	0.3	Set.: 30 s; hard.: 5 (2min)
βTCP/MCPM/ HAP	1.45	Water	1.4	Set.: 1 h; hard.: 2 (24h)
		Gelling CGP (1% C),	1	Set.: 1 h; hard.: 2 (24h)
βTCP/MCPM/ $CaCO_3$	1.41	Water	0.4	Set.: 1 h; hard.: 5 (4h)
		Gelling CGP (1% C)	0.4	Set.: 1 h; hard.: 3 (2h)
βTCP/MCPM/ DCPA	1.00	Gelling CGP (1% C)	0.40	Set.: 1 h; hard.: 5 (1h)
βTCP/Citric ac.	1.50	Gelling CGP (1% C)	0.40	Set.: 20 h; hard.: 5 (24h)
βTCP/MCPM/ Citric ac.	1.45	Gelling CGP (1% C)	0.40	Set.: 3 h; hard.: 5 (24h)
βTCP/MCPM/ NaH_2PO_4	1.45	Gelling CGP (1% C)	0.30	Set.: 1 h; hard.: 3 (24h)

S/L ratio: is given in ml of liquid phase per gram of solid phase.

Ca/P*: Total Ca/P ratio for solid phase.

Hardness: from 0 (liquid) to 5 (very hard).

Table 10

Composition of liquid and solid phases for TCP/MCP based cement-like bio-materials

Mineral Phase	Ca/P	Liquid Phase	L/S ml/g	Observations
βTCP/ DCPA	1.45	Water	0.45	Set.: 5 h; hard.: 3 (24h)
		Gelling CGP (C 1%)	0.45	Set.: 2 h; hard.: 2 (24h)
βTCP/ DCPA	1.45	NaPO4 0.01M	0.40	Set.: 19 h; hard.: 1 (24h)
		Gelling CGP (C 1%), NaP 0.01M	0.40	Set.: 2 h; hard.: 2 (24h)
βTCP/ DCPA/ HAP	1.59	Gelling CGP (C 1%)	1.0	Set.: 2 h; hard.: 2 (24h)
		Gelling CGP (C 1%), NaP 0.01M	1.0	Set.: 2 h; hard.: 2 (24h)
βTCP/ DCPA/ $CaCO_3$	1.59	Gelling CGP (C 1%)	0.40	Set.: 1 h; hard.: 4 (24h)
		Gelling CGP (C 1%), NaP 0.01M	0.40	Set.: 1 h; hard.: 4 (24h)

S/L ratio: is given in ml of liquid phase per gram of solid phase.
Ca/P*: Total Ca/P ratio for solid phase.
Hardness: from 0 (liquid) to 5 (very hard).

TCP/DCP calcium phosphate content

The composition and preparation were identical to those described previously in Example 2a, except that monocalcium phosphate (MCP anhydrous or hydrated) was replaced by di-calcium phosphate (DCP anhydrous or hydrated). One typical example comprises 3.10 g TCP and 0.30 g DCP. Other mineral ingredients may be incorporated in the solid phase: in the Examples (see Table 11), calcium carbonate minerals were added, thus giving carbonated apatites. The Ca/P ratio of compositions was between 1.40 and 1.60.

b) TTCP Cement-like compositions and bio-materials

TTCP/MCP calcium phosphate contents

Liquid phases were composed of pure phosphate aqueous buffer (control), chitosan-GP aqueous systems (see Examples 1),

was ranging from 1.33 to 1.80. Solid TTCP/MCP phases were obtained by dry-mixing, either manual mixing or rotative mixing, of mineral powders. Manual mixing was done with a mortar and pestle. Rotative mixing was performed at low speeds in closed 50cc chambers that contained 10-30cc of mineral materials (~50% free volume).

TTCP/DCP calcium phosphate contents

TTCP/DCP mineral phases were also used to prepare cement-like compositions and bio-materials. TTCP/DCP based phases were prepared from tetra-calcium phosphates (TTCP) and di-calcium phosphates (DCP Anhydrous or Di-hydrated), but optionally also contained other mineral ingredients (carbonated, fluorinated). Powder mixture of TTCP and DCP was equimolar (see Table 12).

Table 12

Composition of liquid and solid phases for TTCP/DCP based cement-like bio-materials

Mineral Phase	Ca/P	Liquid Phase	L/S ml/g	Observations
TTCP/DCPA	1.66	Water	0.60	Set.: 24 h; hard.: 1 (24h)
		Gelling CGP (C 1%)	0.50	Set.: 3 h; hard.: 3 (24h)
TTCP/DCPA	1.66	NaP 0.01M	0.40	Set.: 3 h; hard.: 2 (20h)
		Gelling CGP (C 1%), NaP 0.01M	0.40	Set.: 3 h; hard.: 2 (20h)
TTCP/DCPA/ HAP	1.66	Gelling CGP (C 1%)	0.75	Set.: 2 h; hard.: 5 (5h)
		Gelling CGP (C 1%), NaP 0.01M	1.00	Set.: 2 h; hard.: 3 (24h)
TTCP/DCPA/ $CaCO_3$	1.75	Gelling CGP (C 1%)	0.50	Set.: 19 h; hard.: 2 (24h)
		Gelling CGP (C 1%), NaP 0.01M	0.50	Set.: 19 h; hard.: 2 (24h)
TTCP/DCPA/ Citric ac.	1.66	Gelling CGP (C 1%)	0.30	Set.:1 h; hard.: 6 (1h)
TTCP/DCPA/ NaH_2PO_4	1.66	Gelling CGP (C 1%)	0.80	Set.: 1 h; hard.: 5 (1h)

Table 13

Composition of liquid and solid phases for TTCP/TCP based cement-like bio-materials

Mineral Phase	Ca/P	Liquid Phase	L/S ml/g	Observations
TTCP/ βTCP	1.66	Water	0.45	Set.: 3 h; hard.: 3 (24h)
		Gelling CGP (C 1%)	0.45	Set.: 3 h; hard.: 3 (24h)
TTCP/ βTCP	1.66	NaP 0.01M	0.40	Set.: 19 h; hard.: 2 (24h)
		Gelling CGP (C 1%), NaP 0.01M	0.40	Set.: 19 h; hard.: 1 (24h)
TTCP/ βTCP/HAP	1.66	Gelling CGP (C 1%)	1.0	Set.: 4 h; hard.: 1 (24h)
		Gelling CGP (C 1%), NaP 0.01M	1.0	Set.: 2 h; hard.: 2 (24h)
TTCP/ βTCP/ $CaCO_3$	1.75	Gelling CGP (C 1%)	0.40	Set.: 19 h; hard.: 2 (24h)
		Gelling CGP (C 1%), NaP 0.01M	0.40	Set.: 19 h; hard.: 2 (24h)

S/L ratio: is given in ml of liquid phase per gram of solid phase.
Ca/P*: Total Ca/P ratio for solid phase.
Hardness: from 0 (liquid) to 5 (very hard).

TCP was either α- or β-tricalcium phosphate. Dry mixing was achieved as previously described in Example 2a-2b. Liquid phases were pure water or chitosan-GP aqueous systems. Admixing and homogenization of liquid and solid phases was done as previously reported in Examples 2a-2b.

d) Other Cement-like compositions and bio-materials

Other combinations of calcium phosphate minerals were performed to prepare the solid phase. Some given examples report the use of hydroxyapatite (HAP) crystal or other seeding (see Table 14).

Table 14

Composition of liquid and solid phases for cement-like bio-materials

Mineral Phase	Ca/P	Liquid Phase	L/S ml/g	Observations
βTCP/OCP	1.45	Gelling CGP (C 1%)	1.0	Set.: 1 h; hard.: 3 (24h)
		Gelling CGP (C 1%), NaP 0.01M	1.0	Set.: 1 h; hard.: 3 (24h)
TTCP/OCP	1.66	Gelling CGP (C 1%)	1.20	Set.: 1 h; hard.: 4 (16h)
		Gelling CGP (C 1%), NaP 0.01M	1.20	Set.: 1 h; hard.: 4 (16h)
TTCP/OCP	1.66	Gelling CGP (C 1%)	1.20	Set.: 1 h; hard.: 4 (16h)
		Gelling CGP (C 1%), NaP 0.01M	1.20	Set.: 1 h; hard.: 4 (16h)
MCPM/OCP /HAP	1.25	Gelling CGP (C 1%)	0.9	Set.: 3 h; hard.: 3 (5h)
MCPM/OCP /CaO/HAP	1.45	Gelling CGP (C 1%)	2.4	Set.: 1 h; hard.: 2 (5h)
MCPM/OCP /CaCO3/ HAP	1.46	Gelling CGP (C 1%)	2.0	Set.: 1 h; hard.: 2 (5h)
MCPM/CaO/ HAP	1.56	Gelling CGP (C 1%)	1.4	Set.: < 1 h; hard.: 3 (1h)
		Gelling CGP (C 1%), NaP 0.01M	1.4	Set.: < 1 h; hard.: 3 (1h)
MCPM/CaO/ HAP	1.56	Gelling CGP (C 1%)	1.4	Set.: < 1 h; hard.: 3 (1h)
		Gelling CGP (C 1%), NaP 0.01M	1.4	Set.: < 1 h; hard.: 3 (1h)
MCPM/ $CaCO_3$/HAP	1.48	Gelling CGP (C 1%)	1.0	Set.: 1 h; hard.: 4 (20h)
MCPM/CaO/ $CaCO_3$/HAP	1.47	Gelling CGP (C 1%)	1.4	Set.: 1 h; hard.: 4 (20h)
HAP/CaO/ ZnO/MCPM	1.50	Gelling CGP (C 1%)	1.6	Set.: 1 h; hard.: 4 (20h)

Table 15

Composition of liquid and solid phases for self-setting hybrid compositions and bio-materials (TCP and TTCP based)

#	Solid phase	Liquid phase (% in w/v)	Ca/P	L/S (mL/g)	Setting/Hardening
1	TTCP + MCPM	CGP, C 1%.	1.66	0.55	Suspension with limited homogeneity; Injectability difficult; Setting; Hardening (0-4 hrs). Hard after ageing.
2	TTCP + DCPA + MCPM	CGP, C 1%.	1.50	0.6	Fine & workable slurry; Injectability difficult; Setting Hardening (0-4 hrs). Hard after ageing.
3	TTCP + DCPA + Citric ac.	CGP, C 1%.	1.66	0.32	Fine & workable slurry; Injectability must be rapid; Setting rapid; Hardening (1 min). Hard after ageing.
4	βTCP + MCPM	CGP, C 1%.	1.33	0.3	Fine & workable slurry; Injectability must be rapid; Setting rapid; Hardening (1 min). Hard after ageing.
5	αTCP + MCPM + HA	CGP, C 1%.	1.37	0.7	Suspension with limited homogeneity; Injectability difficult; Setting; Hardening (0-6 hrs). Hard after ageing.
6	αTCP + DCPA + HA	CGP, C 1%.	1.33	0.5	Fine, flowable & workable slurry; Injectable; Setting; Hardening (0-6 hrs). Hard after ageing.
7	αTCP + citric ac. + HA	CGP, C 1%.	1.50	0.4	Fine, flowable & workable slurry; Injectable; Setting; Hardening (0-10 min.). Hard after ageing.

Table 17

Composition of liquid and solid phases for self-setting hybrid alpha-TCP based compositions and bio-materials (changes of apatitic charge)

#	Solid phase (% wt.)	Liquid phase (% in w/v)	Ca/P	L/S (mL/g)	pH range (start. – final)	Setting/Hardening
1	αTCP + citric ac. (11.7%) + CDAc (10.0%)	CGP, Chitosan 1%.	1.50	0.40	4.80 – 5.46	Initial set.: 4 min Final set.: 8-10 min
2	αTCP + citrate (9.0%) + CDAc (10.0%)	CGP, Chitosan 1%.	1.50	0.40	5.76 – 6.60	Initial set.: 6 min Final set.: 30 min
3	αTCP + citrate (11.7%) + CDAc (10.0%)	CGP, Chitosan 1%.	1.50	0.40	5.75 - 6.30	Initial set.: 7 min Final set.: 30 min
4	αTCP + citrate (9.0%) + CDAh (10.0%)	CGP, Chitosan 1%.	1.50	0.40	6.13 – 6.62	Cohesion time: 8 min Initial set.: 9 min Final set.: 33 min Injectability is 100%
5	αTCP + citrate (9.0%) + SHA (10.0%)	CGP, Chitosan 1%.	1.50	0.40	6.02 – 6.59	Cohesion time: 7 min Initial set.: 9 min Final set.: 33 min Injectability is 100%

CDAc: Calcium Deficient Apatite, commercial, Ca/P = 1.5;

CDAh: Calcium Deficient Apatite, home synthesized, Ca/P = 1.5;

SHA: Sintered Hydroxyapatite, commercial;

pH (start. – final): pH after preparation of slurry – pH after setting (>8 hrs)

Table 19

pH change of an alpha-TCP based self-setting Composition (see Fig. 4)

#	Solid phase (charge, % wt.)	L/S = 0.30 mL/g	L/S = 0.40 mL/g	L/S = 0.50 mL/g
1	CDAc 0.0%	pH start: 6.09 pH final: 6.70	pH start: 6.27 pH final: 6.75	pH start: 6.02 pH final: 6.65
2	CDAc 5.0%	pH start: 6.11 pH final: 6.66		
3	CDAc 10.0%	pH start: 6.06 pH final: 6.70	pH start: 5.76 pH final: 6.60	pH start: 5.97 pH final: 6.55
4	CDAc 15.0%	pH start: 6.04 pH final: 6.68		
5	CDAc 20.0%	pH start: 5.84 pH final: 6.62	pH start: 5.76 pH final: 6.56	
6	CDAc 50.0%		pH start: 5.73 pH final: 6.30	

CDAc : Calcium Deficient Apatite, commercial, Ca/P = 1.50;
CT: cohesion time (in min)
In. set.: initial setting (in min)
Fin set.: final setting (in min)
Inj.: injectability (capacity to be injected)
pH (start. – final): pH after preparation of slurry – pH after setting (>8 hrs)

Table 21

Setting and injectability of an alpha-TCP/Calcium Carbonate based self-setting Composition

#	Solid phase Liquid phase* (charge, % wt.)	1 $CaCO_3$ 5.9%	2 $CaCO_3$ 10.0%	3 $CaCO_3$ 9.15%	4 $CaCO_3$ 20.0%
1	αTCP CDAc 15.0% Citrate 9.0% L/S=0.4 mL/g	CT: 9.0 min In. set.: 12.0 min Fin set.: 44.0 min Inj.: 0% pH: 5.7 – 6.6			
2	αTCP CDAc 15.0% Citrate 9.0% L/S=0.4 mL/g		CT: 5.0 min In. set.: 13.0 min Fin set.: 27.0 min Inj.: 100%		
3	αTCP CDAc 15.0% Citric ac. 11.7% L/S=0.4 mL/g			CT: 6.0 min In. set.: 4.0 min Fin set.: 12.0 min Inj.: 5-10%% pH: 4.8 – 6.03	
4	αTCP CDAc 15.0% Citric ac. 11.7% L/S=0.4 mL/g				CT: 5.0 min In. set.: 4.0 min Fin set.: 12.0 min Inj.: 100% pH: 4.7 – 6.3

CDAc : Calcium Deficient Apatite, commercial, Ca/P = 1.50;

CT: cohesion time (in min) In. set.: initial setting (in min)

Fin set.: final setting (in min) Inj.: injectability (capacity to be injected)

pH (start. – final): pH after preparation of slurry – pH after setting (>8 hrs)

(Liquid: chitosan-glycerophosphate-water, chitosan 1% w/v);

features hereinbefore set forth, and as follows in the scope of the appended claims.

a basic character.

8. The composition of Claim 1, wherein said liquid component comprises a first phosphate source selected from the group consisting of $Na_2PO_4C_3H_5(OH)_2$, $Fe_2PO_4C_3H_5(OH)_2$, $K_2PO_4C_3H_5(OH)_2$, $MgPO_4C_3H_5(OH)_2$, $MnPO_4C_3H_5(OH)_2$, $Ca_2PO_4C_3H_5(OH)_2$, $Na_2PO_7C_3H_7$, $Na_2PO_7C_4H_7$, $K_2PO_7C_4H_7$, $NaPO_7C_4H_8$, $K_2PO_7C_4H_8$, $Na_2PO_8C_5H_9$, $K_2PO_8C_5H_9$, $NaPO_8C_5H_{10}$, $KPO_8C_5H_{10}$, $Na_2PO_9C_6H_{11}$, $NaPO_9C_6H_{12}$, $K_2PO_9C_6H_{11}$, $KPO_9C_6H_{12}$, $Na_2PO_8C_6H_{13}$, $K_2PO_8C_6H_{13}$, $NaPO_8C_6H_{14}$, $KPO_8C_6H_{14}$, $Na_2PO_9C_6H_{12}$, $K_2PO_9C_6H_{12}$, $NaPO_9C_6H_{13}$, $KPO_9C_6H_{13}$, $Na_2PO_8C_{10}H_{11}$, $K_2PO_8C_{10}H_{11}$, $NaPO_8C_{10}H_{12}$, $KPO_8C_{10}H_{12}$ and the like, or a derivative thereof.

9. The composition of Claim 1, wherein said monophosphate salt is a sodium, magnesium, potassium, ferric and/or calcium alpha- or beta-glycerophosphate salt, or a mixture thereof.

10. The composition of Claim 1, wherein said monophosphate salt is glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate or fructose-6-phosphate salt, or a mixture thereof.

11. The composition of Claim 1, wherein said liquid component has a pH between 6.8 and 7.2.

12. The composition of Claim 1, wherein said liquid component has a viscosity superior to 200 mPa.s.

13. The composition of Claim 1, wherein said liquid component further comprises at least one other water-soluble polymer selected from the group consisting of polypeptides, cellulosics and synthetic polymers, including methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl propylcellulose, hydroxymethyl propylcellulose, poly(ethylene oxide), poly(propylene oxide), poly(ethylene glycol), poly(vinylpyrrolidone), poly(vinyl alcohol), and derivatives thereof.

23. The composition of Claim 1, wherein said powder component comprises tetracalcium phosphate and dicalcium phosphate.

24. The composition of Claim 1, wherein said powder component comprises tetracalcium phosphate and monocalcium phosphate.

25. The composition of Claim 1, wherein said powder component comprises less than 40% wt. of an apatitic calcium phosphate.

26. The composition of Claim 1, wherein said powder component further comprises at least one fluoride selected from the group consisting of NaF, Na_2Si_6F, KF, KSi_6F, CaF_2, MgF_2, ZnF_2, and sodium fluorophosphates, and the like, or derivatives thereof.

27. The composition of Claim 1, wherein said powder component further comprises at least one carbonate selected from the group consisting of Na_2CO_3, $CaCO_3$, K_2CO_3, $MgCO_3$, $ZnCO_3$, $Ca_9K(PO_4)_5(CO_3)_2$, $Ca_{8.5}Na_{1.5}(PO_4)_{4.5}(CO_3)_{2.5}$, $Ca_9(PO_4)_{4.5}(CO_3)_{1.5}$, and the like.

28. The composition of Claim 1, wherein said powder component comprises a strontium salt including strontium carbonate.

29. The composition of Claim 1, wherein said powder component comprises at least one calcium phosphate selected from the group consisting of fluoride, strontium, carbonate, magnesium, zinc, and barium containing calcium phosphates.

30. The composition of Claim 1, wherein said powder component comprises at least one inorganic salt including sodium phosphates and disodium glycerophosphate, or the like.

31. The composition of Claim 1, wherein said powder component comprises at least one organic salt including oxalate, citrate, malate, gluconate, lactate, lactobionate, or the like.

or diaphysis of a bone, said composition setting *in situ* into a filling hardened material.

41. Use of the composition of Claim 1 for injection into a fractured bone, between the bone fragments of fractured bone, said composition setting *in situ* into a filling hardened material.

42. An injectable self-setting composition comprising:

a) a liquid component, free of insoluble material, comprising, an organic and/or inorganic acid, a partially N-deacetylated chitosan and/or a collagen, and a glycerophosphate; said liquid component having a pH ranging from 6.5 to 7.4, said liquid component having an endothermally gelling character, said partially N-deacetylated chitosan having a final concentration ranging between 0.5 to 3.0% w/v, and said glycerophosphate salt having a final concentration ranging between 1.0 to 10.0% w/v, and

b) a powder component comprising a dry mixture of a tricalcium phosphate with a calcium deficient apatite or an octacalcium phosphate, and with at least one of an inorganic salt, an organic salt, an organic acid source and an organic compound,

wherein when said components of step a) and b) are intimately and uniformly mixed together, said components of step a) and b) form an injectable thermo-setting slurry, said slurry when heated turns into a solid material.

43. An injectable self-setting composition comprising:

a) a liquid component, free of insoluble particle, comprising an organic and/or inorganic acid, a partially N-deacetylated chitosan and/or a collagen, and a glycerophosphate; said liquid component having a pH ranging from 6.5 to 7.4, said liquid component having an endothermally gelling character, said partially N-deacetylated chitosan has a concentration ranging between 0.5 to 3.0% w/v, and said glycerophosphate salt has a concentration ranging between 1.0 to 10.0% w/v, and

50. A composition as described in Claim 42 or 43, which further comprises a strontium containing compound.

51. A composition as described in Claim 42 or 43, which further comprises a carbonate containing compound.

52. A composition as described in Claim 42 or 43, which further comprises a fluoride containing compound.

53. Use of a composition as described in Claim 42 or 43 for injection into a defect, cavity or substance of a mammalian or human hard-tissue, said composition setting *in situ*.

54. Use of a composition as described in Claim 42 or 43, for injection in association with a permanent or biodegradable fixative device, said composition setting *in situ*.

55. A method of preparation of an injectable self-setting composition, said method comprising the step of admixing a water-based liquid component comprising at least one cationic polymer and one mono-phosphate salt with a powder component comprising at least two calcium phosphate sources selected from apatites and apatitic calcium phosphates, octacalcium phosphates, amorphous calcium phosphates, tetracalcium phosphates, tricalcium phosphates, dicalcium phosphates and monocalcium phosphates, wherein said liquid component comprising at least one cationic polymer and one mono-phosphate salt; said liquid component having a pH ranging from 6.5 to 7.4, said liquid component having an endothermally gelling character and being free of insoluble particles, said admixing thus forming an injectable thermo-setting slurry, said slurry when heated turns into a solid material.

56. A method of preparation of an injectable self-setting composition, said method comprising admixing a liquid component, free of insoluble material, comprising, an organic and/or inorganic acid, a partially N-deacetylated chitosan and/or a collagen, and a glycerophosphate, with a

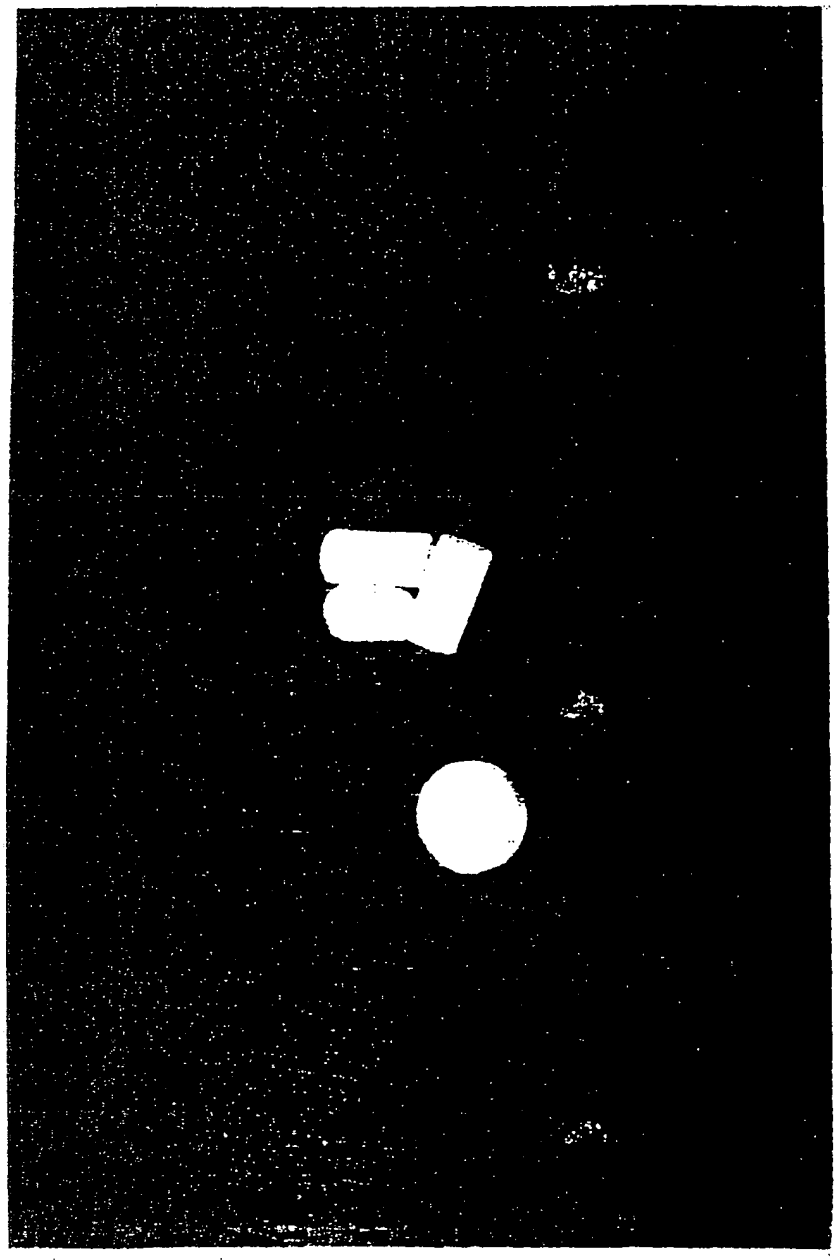

Fig - 1

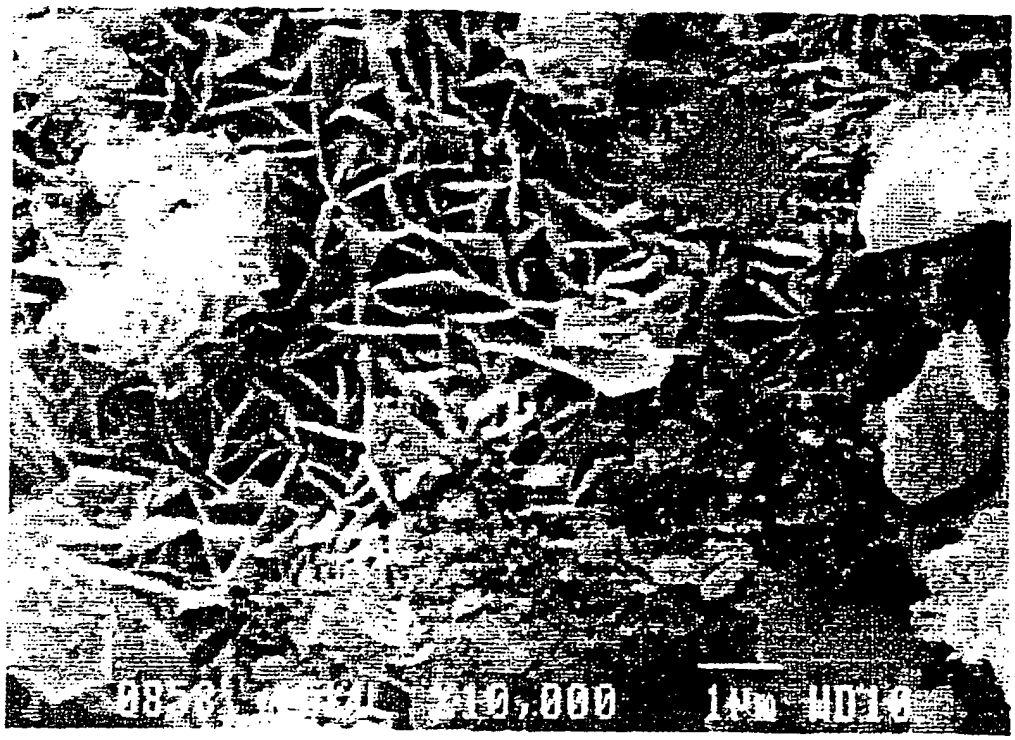

FIG 3

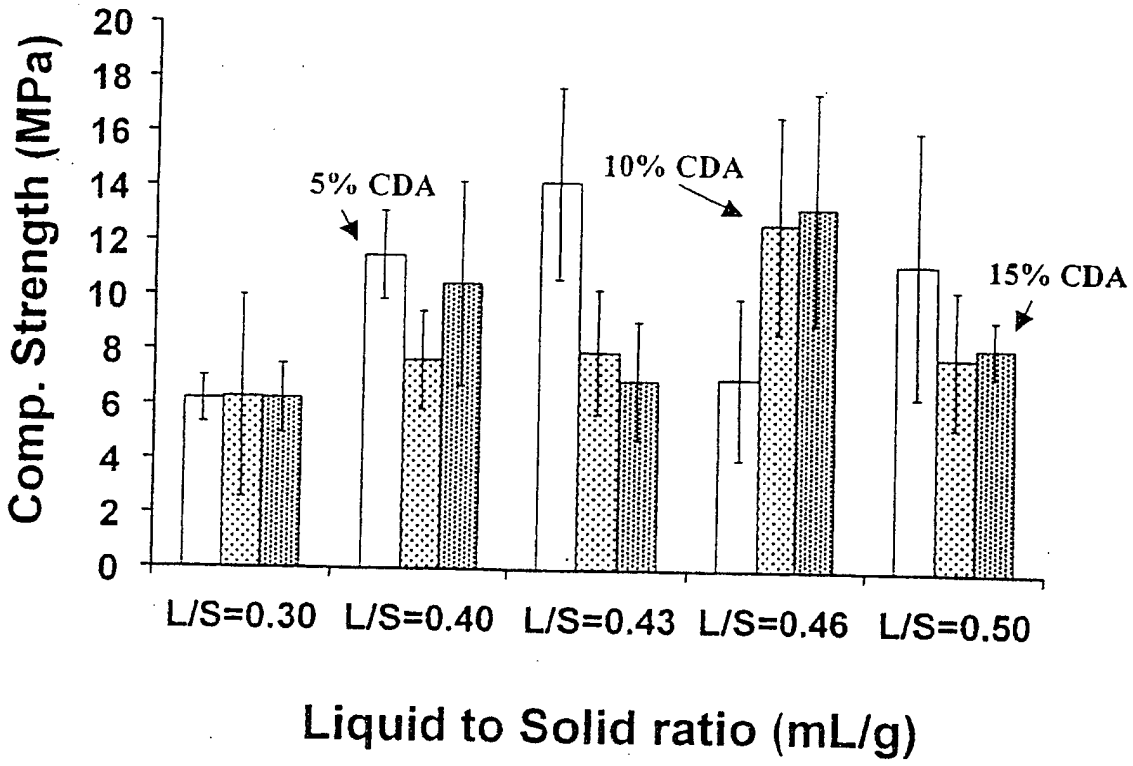
Comp. Strength (MPa)
20
18
16
14
12
10
8
6
4
2
0
5% CDA
10% CDA
15% CDA
L/S=0.30
L/S=0.40
L/S=0.43
L/S=0.46
L/S=0.50
Liquid to Solid ratio (mL/g)

Fig. 5